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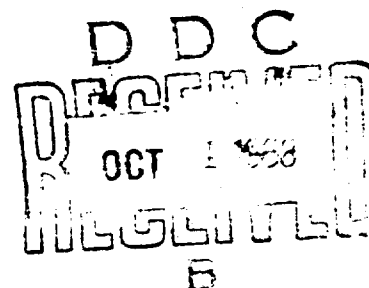
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## EXPERIMENTAL AIRBORNE TULAREMIA

[Following is the translation of an article by F. Kintera and Vl. Vortel, Military Medical Scientific-Research Institute and the Institute for the Advancement of Doctors imeni Purkinye, Karlov University, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 9, 1966, pages 62-66. It was submitted on 8 July 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The F. tularensis aerosol was obtained in an experimental chamber with a dynamic system. The necessary physical parameters - reduced pressure, temperature, relative humidity - could be regulated, therefore in all the tests they were almost constant. All the operations were completely safe in a 400 liter capacity experimental chamber at a constant pressure, reduced to 8 mm water column, air temperature 18-20°, and relative humidity 85%.

Under a pressure of 0.5-1 atmospheres the suspension of microbes from a graduated container entered a straight metallic sprayer with a mixer. An infectious aerosol, made up of particles 0.5-4.5 microns in size, was developed in the sprayer. The infectious aerosol was mixed with separately prepared air, which with the help of the mixer was forced into the chamber at approximately 90 liters/min. The samples of the infectious aerosol were taken with the help of a flow meter, placed in control impingers with the appropriate medium, and a calculation of the inhalation dose made (Figure 1).

We used the strains F. tularensis No. 2713 (virulent strain, isolated in the Republic of Czechoslovakia) and No. 15 (Elbert and Gayskiy vaccine strain with attenuated virulence). The strains were incubated on platelets made of 2% blood agar (rabbit blood) with 0.25% sodium thioglycollate (Difco medium).

Guinea pigs (average weight 320 g) breathed in the infectious aerosol for 10 minutes. The magnitude of the inspired dose was measured by the method of Guyton - by means of oscillographic determination of the volume of air, inspired by the guinea pig, on the basis of the quantitative titration of the suspension and the concentration of sprayed aerosol in 1 liter in the experimental chamber. The lethal dose of strain No. 2713 equaled  $6 \cdot 10^2$  microbial cells. Following inhalation of this dose the guinea pigs died in the course of 9 days.

For the purpose of studying the multiplication of microbes in the organism 3 pigs were autopsied daily following the inhalation. The quantitative method was used for calculating the amount of *Francisella*

in the individual organs (lungs, tracheobronchial node, spleen, liver) and in the blood. The presence of causative agent was determined both per 1 g of organ weight and for the whole organ.

For the purpose of studying the multiplication of the microbes in immunized animals, 120 guinea pigs were inoculated with a live vaccine strain of *Francisella* (No. 15). It was administered intracutaneously in a dose of  $94 \cdot 10^4$  microbial cells. After 5 weeks the immunized pigs were subjected to the action of 1 and 100 inhalation doses of the infectious aerosol. The dynamics of the accumulation of *F. tularensis* in the separate organs of normal and immunized guinea pigs are presented in Figure 2.

The tularemia bacilli were encountered in the lungs of normal guinea pigs from the 1st through the 9th day. In 24 hours the number of microbes increased by approximately 1 order. With an inhalation dose of 1-100 Dlm the number of microbes in the lungs of immunized guinea pigs was insignificant. Up to the 5th day it fluctuated within the limits of  $10^2 - 10^3$  without a tendency for significant multiplication. With an inhalation dose equal to 1 Dlm, after the 5th day it was not possible to detect microbes by means of inoculation. With a dose 100 times greater the microbes survived in the lungs up to the 7th day. In experiments with the same inhalation doses the microbes were not seeded out from the lungs after the 7th day.

Seedings from the bifurcate nodes of normal guinea pigs were positive during the period from the 2nd to the 9th day. The number of *Francisella* during the 9 days increased from zero values up to  $10^{10}$  per 1 gram of organ weight. With an inhalation dose equal to 1 Dlm, on the 2nd day following injection we detected *Francisella* in the lymph nodes of immunized guinea pigs. Multiplication of the causative agent in the nodes was not observed, either, on the other hand their number decreased up to the 7th day and later the nodes remained practically sterile. With an inhalation dose of 100 Dlm no essential changes were detected in the regional lymph nodes of immunized guinea pigs. The maximum number of *Francisella* ( $10^3$  per 1 gram of lymphatic tissue) in the regional lymph nodes correspond to the inspired dose.

In the spleen of normal guinea pigs the microbes appeared between the 3rd and 9th day with an expressed tendency for multiplication mainly beginning with the 6th day. After a resolving *F. tularensis* inhalation dose of 1-100 Dlm it was not possible to isolate the causative agent from the spleen of immunized pigs.

In seedings from the liver of normal pigs a positive result was observed between the 3rd and 9th day. The number of *Francisella* increased by 4 logarithm exponents (up to a value of  $10^8$  per 1 gram of weight), while from the immunized guinea pigs the tularemia causative agent was not seeded out.

From the blood of normal guinea pigs *Francisella* was seeded out only on the 5th day. A blood culture was obtained up to the 9th day. In seedings from the blood of immunized guinea pigs, infected with 1-100 Dlm, negative results were always obtained.

The results attested to the sufficiently expressed immunity of guinea pigs, immunized with Soviet vaccine strain *F. tularensis* No. 15, to inspiration of 1 and 100 Dlm of a virulent strain. It should be added that after penetration into the lower respiratory tract of normal animals, the inspired *Francisella* multiplied strongly and caused a specific inflammation of the lungs: 24 hours after inspiration there was expressed focal hyperemia of the pulmonary parenchyma and edema, exudation of the white blood corpuscles in groups of alveoli and the formation of miliary foci of pneumonia. Over a period of 3-4 days the injured lung usually turned out to be permeated with miliary yellow foci, which is a number of places fused into large foci. The accumulation of pathologic foci around the bronchi and vessels was conspicuous.

Foci, made up of white blood corpuscles, and later - of histiocytes, developed in the interstitial space around the bronchi and vessels. These foci enlarged and led to the ulceration of the bronchial wall. Exudate poured through the ulcers into the lumen of the neighboring bronchus (auto-infection). Then the exudate filled the bronchus and its peripheral branches. The focus acquired the form of branching horns.

In the presence of developing pneumonia the causative agent was found not only isolatedly in the exudate breaking up in the alveoli, but turned out to be absorbed by histiocytes, groups of which could be seen in the fissures around the large vessels, bronchi, and also in the subpleural space and in those sectors where the inflammatory reaction was still absent or was weakly expressed.

From the lungs the microbes used the lymphatic route to penetrate into the regional lymph nodes, where they multiplied. In the nodes an expressed exudation of the white blood corpuscles was observed, mainly in peripheral cavities, as well as the formation of abscesses, which bordered the zones of multiplying histiocytes. This zone could be of a various width. The tracheobronchial nodes and the nodes in the porta of the lungs were most strongly affected. The nodes situated below were usually affected to the same degree as the tracheobronchial; in the upper nodes there were considerably less morbid foci.

*Francisella* penetrated into the blood stream by way of the lymphatic route through the thoracic duct, and from there by way of the blood vessels to other organs.

Qualitatively the same changes as in the nodes were observed in the spleen beginning approximately with the 4th day after inhalation infection.

In the liver we found diffuse necrotized miliary foci beginning with the 4th-5th day after infection. The foci were made up of necrotic liver cells and broken up leukocytes.

In the lungs of the immunized guinea pigs the pathohistological picture was principally different from the picture observed in the normal pigs. On the 1st day after infection the multiplication of lymphoid cells in the fissures around the vessels and bronchi was observed. It can be conjectured that their multiplication took place already during the period of immunization. The expressed multiplication of histiocytes both in the enlarged septa and the pulmonary alveoli was also detected. The process had a focal nature. Leukocytes were never present in them. In the nodes of the mediastinum we found an expressed lymphoid hyperplasia without acute focal changes.

There were no focal changes in the spleen or in the liver; in the spleen only an insignificant multiplication of reticular cells was observed.

The pathohistological changes during inhalation tularemia are presented in Figure 3.

#### Conclusions

1. A study was made of the histopathological changes and also of the accumulation of microbes in guinea pigs during the process of inspiring F. tularensis in an experimental dynamic chamber for inhalation of an infectious aerosol.

After a certain period of time, in the normal guinea pigs the number of microbes reached high indices, particularly in the lungs, lymph nodes, spleen and liver.

2. In guinea pigs, actively immunized subcutaneously with Fracisella (5 · 10<sup>5</sup> microbial cells) and subjected to treatment with an infectious aerosol containing F. tularensis No. 2713 in a quantity of 1 and 100 Dlm, the proliferation bore a different nature - the infectious agent penetrated only the regional lymph nodes. ( )

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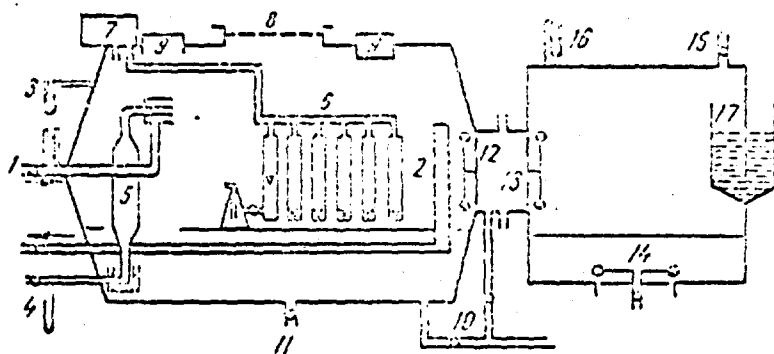


Figure 1. Arrangement of the experimental dynamic chamber.

1 - inflow of air; 2 - outflow of air; 3 - pressure gauge; 4 - air under pressure; 5 - atomizer; 6 - tube gauges; 7 - air pump; 8 - aperture; 9 - lamps; 10 - inflow of steam under pressure; 11 - outflow of condensate; 12 - main closing valve for the chamber; 13 - main closing valve for the movable side; 14 - closing valve for the run-off with mechanized disinfection; 15 - outflow of air into stove for burning; 16 - pressure gauge; 17 - liquid filter with disinfecting solution.

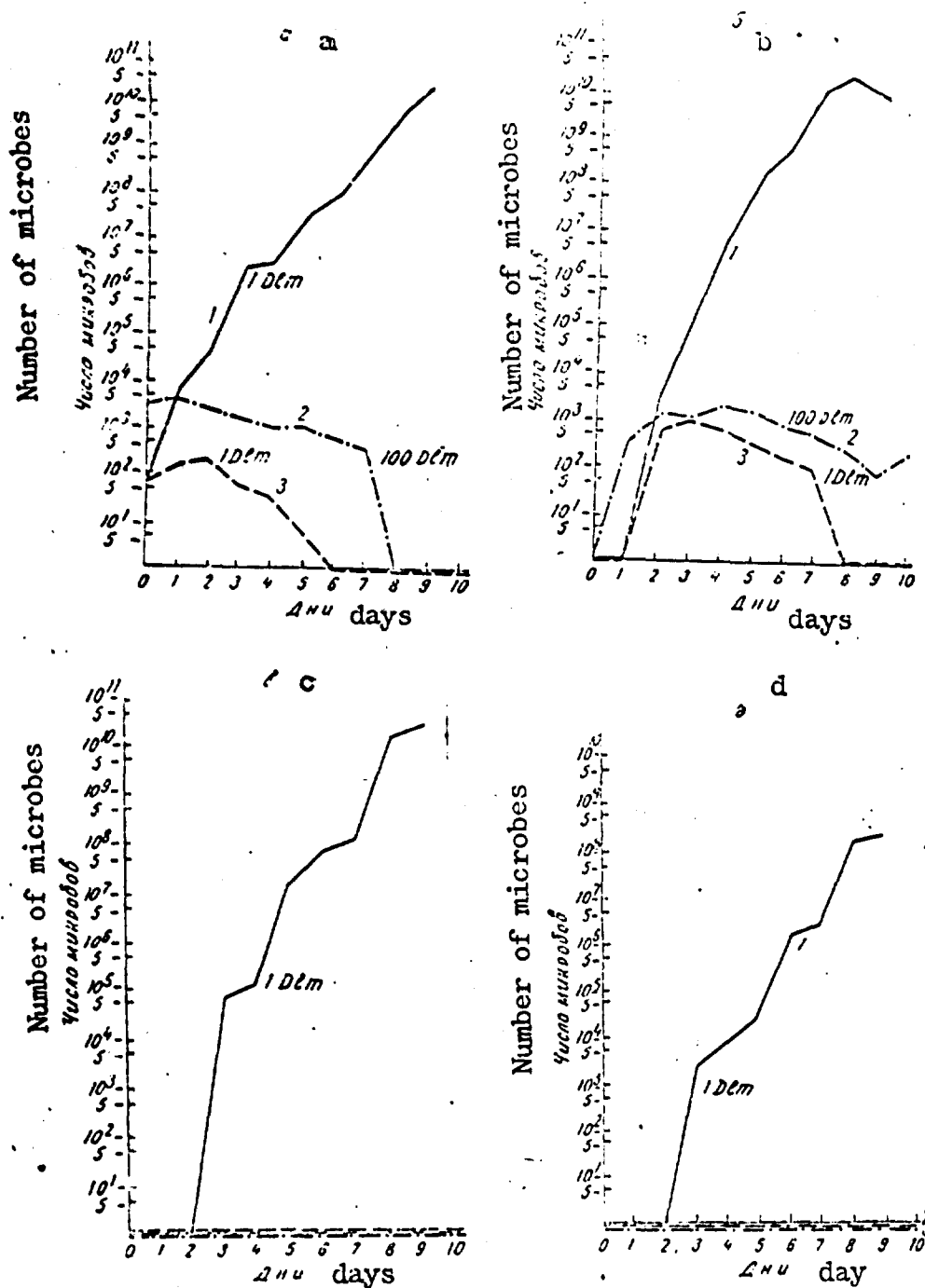


Figure 2. Dynamics of multiplication of *F. tularensis* in various organs in normal and immunized guinea pigs.

a - lungs; b - lymph node; c - spleen; d - liver; 1 - normal pigs; 2 and 3 - immunized pigs. Inhalation infection with 1 and 100 Dlm.

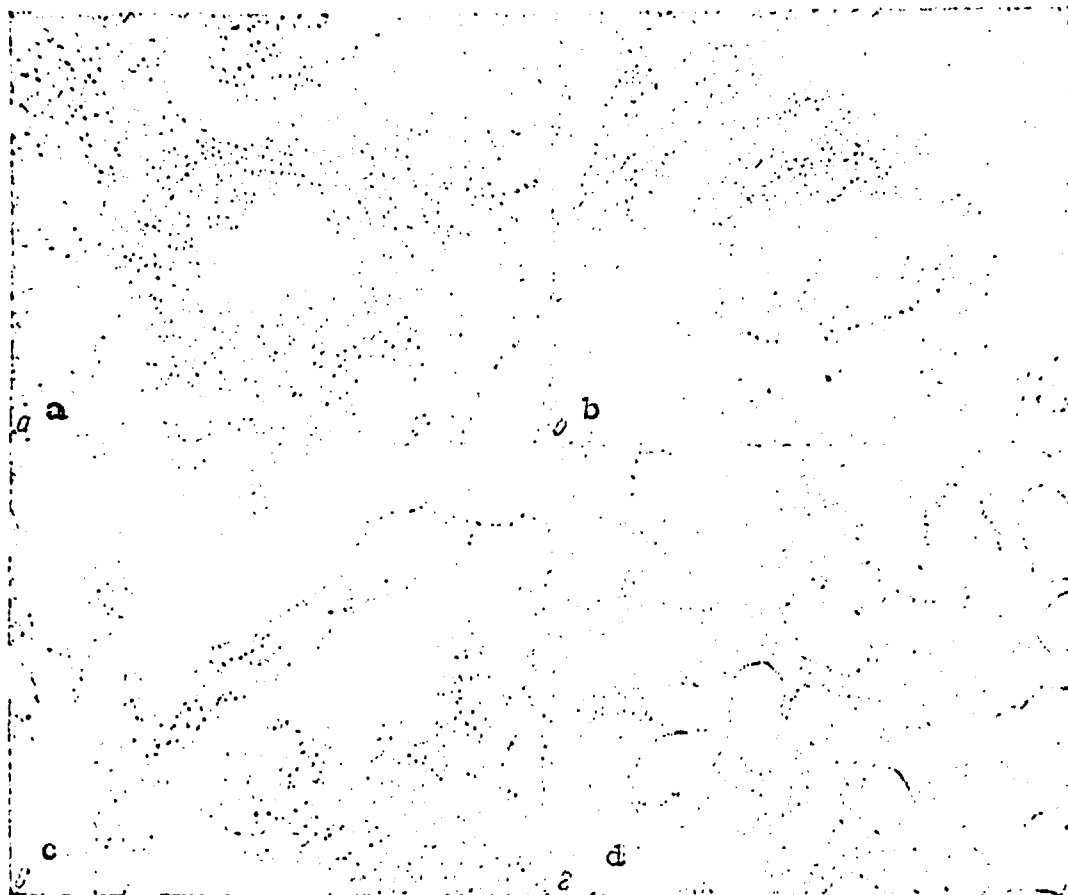


Figure 3. Histopathological changes during inhalation tularemia (stained with hematoxylin-eosin).

- a - inflammatory focus in the septum between the bronchi and vessel (guinea pig No 26 after 72 hours after infection; mag. 100x);
- b - break in the inflammatory focus in the lumen of the bronchus.
- c - ulceration of the bronchial wall, lumen of the bronchus partly surrounded by inflammatory foci (guinea pig No 29 on the 5th day after infection, mag. 100x).
- d - thickened pulmonary septa, infiltrated by multiplying lymphocytes and histiocytes (guinea pig No 36, immunized 4 weeks prior to the experiment, subsequently infected and sacrificed 5 days after infection); mag. 100x.